

## AMENDMENTS TO THE SPECIFICATION

At page 1, below the title, please add the following new heading and paragraph:

### **RELATED APPLICATIONS**

This application is a continuation of U.S.S.N. 09/174,493, filed October 15, 1998, which claims the benefit of provisional applications U.S.S.N. 60/087,216, filed May 29, 1998, and U.S.S.N. 60/062,076, filed October 15, 1997, the contents of which are hereby incorporated by reference in their entirety.

On page 13, please replace the first full paragraph with the following amended paragraph:  
Figure 1. Primary Structure Alignments of *p53*, *p73*, and *p63*.

Human *p53*, human *p73* $\beta$ , human *Tap63* $\gamma$  are presented, with residues identical to *p53* shaded in gray, and remaining consensus residues shaded in black (SEQ ID Nos: 25, 26, 15, and 21).

On pages 13-14, please replace the paragraph bridging pages 13-14 with the following amended paragraph:

Figure 2. Genomic Origin and Diversity of *p63* Isoforms

(A) Schematic of human *p63* gene structure highlighting positions of exons (coding sequences in black), the two promoters in exon one (black arrow) and exon 3' (gray arrow), and the major post-transcriptional splicing events which give rise to the major *p63* isoforms.

(B) Domain structure of *p53*, *p73* $\alpha$  and  $\beta$ , and the major *p63* isoforms, *Tap63* $\alpha$ ,  $\beta$ , and  $\gamma$ , and  $\Delta Np63$  $\alpha$ ,  $\beta$ , and  $\gamma$ , highlighting regions involved in transactivation (TA), DNA binding, and oligomerization (oligo). White box denotes 39aa N-terminal extension unique to TA\**p63*. Gray box represents 14aa unique to  $\Delta Np63$ .

(C) Sequence alignment of N-termini of murine and human *p63* including that found in TA\**p63*, TAp63, and  $\Delta Np63$  (SEQ ID Nos: 45, 46, and 47).

(D) Alignment and comparison of the human *p63* $\alpha$ ,  $\beta$ , and  $\gamma$  C-terminal sequences (SEQ ID Nos: 48, 49, and 50).

On page 102, please replace the second full paragraph with the following amended paragraph:

It has been ~~observed~~ observed that the intron-exon organization was conserved between p73 and p53 (Kaghad et al., 1997), and from known exon and intron sizes for these two genes, it was possible to identify new members of this gene family using a PCR-based strategy of amplifying two exons in a conserved domain and their intervening intron. Sequence similarity in exonic regions would demonstrate a related gene, while differences in size and/or sequence or introns from p53 and p73 would indicate a novel family member. Non-degenerate and degenerate primers were designed based in sequence homology among p53 and p73 cDNAs from various species. Primers (5'-GGCCTCGAGTACAAITWCATGTGTAAAYAG (SEQ ID Nos: 27) and 5'GGCATCGATTCTCTTCCAGGGCAAGCACA (SEQ ID Nos: 28)), designed to anneal to regions in exon 7 and exon 8, respectively, of p73 and p53, were used to amplify products from human and mouse total genomic DNA with the following conditions:

***pre-PCR***: 80°C 2 min, add TAQ polymerase, 94°C 5 min.

***'Touchdown PCR'***: 94°C 1 min, 65°C 1 min,, 72°C 2 min for 3 cycles:

94°C 1 min, 64°C 1 min, 72°C 2 min for 3 cycles; 94°C 1 min, 63°C 1 min, 72°C 2 min for 3 cycles; 94°C 1 min, 62°C 1 min, 72°C 2 min for 2 cycles; 94°C 1 min, 61°C 1 min, 72°C 2 min for 2 cycles; 94°C 1 min, 60°C 1 min, 72°C 2 min for 2 cycles; 94°C 1 min, 59°C 1 min, 72°C 2 min for 2 cycles; 94°C 1 min, 58°C 1 min, 72°C 2 min for 20 cycles; 72°C 7 min

On page 106-107, please replace the paragraph bridging pages 106-107 with the following amended paragraph:

5' Rapid Amplification of cDNA Ends (RACE) was used to obtain further sequence information on p63 not contained within the murine genomic clone. Total RNA was isolated from c15 embryos lacking both p53 and p73, generated from mice bearing targeted mutations in both genes, and used as the template in a first stand cDNA synthesis reaction with a murine p63-specific primer (5'-GGCATCGATGAACTCACGGCTCAGCTC (SEQ ID Nos: 29)). An 'adapter' primer (5'-TTTAGTGAGGGTTAATAAGCGGCCGCGTCGTGACTGGGAGCGC (SEQ ID Nos: 30)) was then ligated to the cDNA product using T4 RNA ligase. PCR was subsequently performed on the ligation product using primers (5'-

GCCCTGGAGGCGGCCGCTTATTAACCCTCAC (SEQ ID Nos: 31) and 5'-GGCATCGATGTAGACAGGCATGGCACG (SEQ ID Nos: 32) with the conditions described in I. An approximately 610bp amplicon was generated, subcloned into pCDNA3 vector, and sequenced in its entirety. The 5'RACE product yielded a sequence corresponding to a N-terminal truncated form of murine p63.

On page 107, please replace the first full paragraph with the following amended paragraph:

A bacterial plasmid cDNA library was constructed from mRNA isolated from e15 embryos lacking both p53 and p73, described above, and screened for p63 cDNAs. Hybridization screens were done essentially as described in V., using a probe, corresponding to exons 5 to 9 of p63, generated by RT-PCR on total p73<sup>-/-</sup>;p53<sup>-/-</sup> mouse RNA with primers (5'-GGGCTCGAGCTGAAGAAGCTGTACTGC (SEQ ID Nos: 33) and 5'-GGGATCGATCTCCGTTTCTTGATGGAA (SEQ ID Nos: 34)). Three clones were identified and sequenced in their entirety. These corresponded to three different, full-length splice variants of murine p63.

On page 114, please replace the second full paragraph with the following amended paragraph:

EMSAs were performed essentially as described in Yang, A. et al., (1998), Mol Cell 2, 305-316. Briefly, human 293 kidney cells were transfected with p53, p63, and GFP expression vectors, as indicated in Figure 25, using the calcium phosphate transfection method previously described (Heald et al., 1993, Cell 74, 463-474.; Yang et al., 1998). Cells were lysed in 150 ml detergent lysis buffer (50mM Tris pH 8, 150 mM NaCl, 0.1% Triton X-100) ~24 hrs after transfection. Lysates were then incubated for 1 hr at room temperature with 100pM <sup>32</sup>P radiolabeled, double-stranded oligonucleotides in binding buffer (16mM Hepes-KOH pH 7.5, 60 mM KCl, 30 mM NaCl, 10% glycerol, 1mM dithiothreitol, 10 mg/ml BSA). The following oligonucleotides were used, with annealing of oligonucleotide pairs performed prior to incubation with lysate extracts above.

PG: 5'-CCTGCCTGGACTTGCTGG (SEQ ID Nos: 35) + 5'-CCAGGCAAGTCCAGGCAGG (SEQ ID Nos: 36).

WAF: 5'-GAACATGTCCCAACATGTTG (SEQ ID Nos: 37) + 5'-  
CAACATGTTGGGACATGTTC (SEQ ID Nos: 38).

MG: 5'-CCTTAATGGACTTTAATGG (SEQ ID Nos: 39) + 5'-CCATTAAAGTCCATTAAGG  
(SEQ ID Nos: 40).

On page 115, please replace the second full paragraph with the following amended paragraph:

Total RNA was isolated from tissues and cell lines using RNeasy, dissolved in 10mM Tris pH8, 1mMEDTA (TE), and quantified using ultraviolet absorption at 260 nm. RT-PCR reactions were performed with the One-Step RT-PCR kit (Gibco-BRL), using 0.25 ug total RNA in 25 ul reactions under the following conditions: 50°C 30 min; 94°C 2 min; 94°C 30 sec, 52°C 30 sec, 72°C 1 min for 40 cycles; 72°C 5 min. The following primers were used: human p63 TA-specific reaction: 5' - ATGTCCCAGAGCCACACAG (SEQ ID Nos: 41) and 5' - AGCTCATGGTTGGGGCAC (SEQ ID Nos: 42); human p63 ΔN-specific reaction: 5' - CAGACTCAATTTAGTGAG (SEQ ID Nos: 43) and 5' - AGCTCATGGTTGGGGCAC (SEQ ID Nos: 44).